Molecular diagnosis of minimal residual head and neck cancer and field cancerization

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Summary

In the introduction (**Chapter 1**) it is outlined that despite significant advances in treatment modalities, the 5-year survival rate of HNSCC patients has not significantly improved [1]. An explanation for this finding is the high frequency of locoregional relapse, second primary tumors and distant metastases [2]. Approximately half of locoregional relapses in patients with histopathologically tumor-free surgical margins are due to minimal residual cancer (MRC): residual cancer cells that are not detected by routine histological examination [3]. The remaining locoregional relapses are in fact new tumors arising in a preneoplastic field: second field tumors (SFTs) [3-5]. The poor survival rates can also be explained by the fact that two-third of patients presents with advanced stage of disease [6], implicating a five-year survival rate of approximately 30% [7].

Aim of this thesis was to develop a method for molecular detection of MRC and high-risk preneoplastic fields. Furthermore, a (non-invasive) screening approach was developed and assessed for detection of preneoplastic fields in risk groups for oral cancer.

In **Chapter 2** a new approach for molecular detection of MRC in deep surgical margins of HNSCC was developed: a qRT-PCR test using hLy-6D, a squamous cell-specific antigen, as molecular marker. The preliminary data of this approach are promising and show a significant difference between the group of patients with histopathologically tumor-positive surgical margins and the non-cancer control group, and the group of patients with histopathologically tumor-free surgical margins.

In **Chapter 3** it is shown that the presence of hLy-6D qRT-PCR positive margins is not significantly related to the development of locoregional relapse, disease-free survival and overall survival. A major problem in the assay appeared to be a high rate of false-negative findings, probably due to sampling error. In contrast to the molecular diagnostic MRC test, the histopathologic parameters vasoinvasive growth and infiltrative growth appeared to be significant prognosticators for locoregional relapse and distant metastasis-free survival.

To overcome the limitations of the hLy-6D qRT-PCR test other target genes are analyzed in **Chapter 4**. Furthermore, in the previous test we noticed problems with normalization, therefore, housekeeping genes are analyzed as well. The molecular marker SCCA seems promising because it shows a very high expression in HNSCC tumors and no expression in control tissue samples, however, the adapted test lacks sensitivity and specificity for MRC detection in HNSCC patients.

In **Chapter 5** it is demonstrated that the presence of a preneoplastic field in surgically treated HNSCC patients is a risk factor for local relapse. LOH at chromosome 9p and presence of immunohistochemical p53-positive surgical margins are significant prognosticators for malignant progression of preneoplastic fields..

In **Chapter 6** a non-invasive screening method to detect preneoplastic fields in the oral cavity was developed. A first analysis was performed on 25 leukoplakia patients and 20 control subjects are shown and appeared to be promising with a specificity of 100%, a sensitivity of 78% and a positive predictive value of 100%.

In **Chapter 7** molecular risk factors for prediction of malignant progression of oral precancer and the potential of the non-invasive test, described in chapter 6, was analyzed. LOH at chromosome 9p and *TP*53 mutations in leukoplakia biopsies appeared to be significant predictors of oral cancer development. It was shown that the non-invasive test is feasible and with high positive predictive value. However, the sensitivity appeared to be relatively low, probably due to hyperkeratosis of leukoplakia lesions. Therefore, the non-invasive test is not suitable for risk assessment in oral leukoplakia lesions, but probably it is suitable in non-visible preneoplastic lesions.

In **Chapter 8** the data presented in this thesis are discussed in a larger perspective.

Reference List

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